

Appendix I—MARKED UP VERSION

Appendix I
Marked-Up Version of Claims
Response to 03/14/02 Office Action
13761-7001 – Due September 14, 2002

In The Claims

14. (Amended) The method of claim ~~13~~ 21 further comprising contacting the subject's a sample of the subject's nucleic acid comprising the MnSOD gene with a probe or primer which can hybridizes to the polymorphic a region of the MnSOD gene encoding the MTS~~mitochondrial targeting sequence of the MnSOD gene~~, said polymorphic region including nucleotide 351 of SEQ ID NO:1.
15. (Amended) The method of claim ~~13~~ 31, wherein determining whether an allele of the MnSOD gene in the subject comprises a mutation in the coding region for the MTS~~the identity of the allelic variant comprises determining the identity of at least one nucleotide of the polymorphic region encoding the MTS~~.
16. (Amended) The method of claim ~~31~~ 43, wherein determining whether an allele of the MnSOD gene in the subject comprises a mutation in the coding region for the MTS~~the identity of the allelic variant comprises performing a restriction enzyme site analysis~~.
17. (Amended) The method of claim ~~13~~ 31, wherein determining whether an allele of the MnSOD gene in the subject comprises a mutation in the coding region for the MTS~~comprises performing at the identity of the allelic variant is carried out by single-stranded conformation polymorphism analysis~~.
18. (Amended) The method of ~~claim 13~~ claim 31, wherein determining whether an allele of the MnSOD gene in the subject comprises a mutation in the coding region for the MTS~~comprises performing at the identity of the allelic variant is carried out by allele specific hybridization~~.
19. (Amended) The method of claim ~~13~~ 31, wherein determining whether an allele of the MnSOD gene in the subject comprises a mutation in the coding region for the MTS~~comprises performing at the identity of the allelic variant is carried out by primer specific extension~~.

Appendix I—MARKED UP VERSION

20. (Amended) The method of claim ~~1331~~, wherein determining whether an allele of the MnSOD gene in the subject comprises a mutation in the coding region for the MTS ~~comprises performing the identity of the allelic variant is carried out by an~~ oligonucleotide ligation assay.
21. (Amended) The method of claim ~~1331~~, wherein the MnSOD gene is a human MnSOD gene.
22. (Amended) The method of claim ~~1314~~, wherein the probe or primer has a nucleotide sequence from about 15 to about 30 nucleotides.
23. (Amended) The method of claim ~~1331~~, wherein the probe or primer is labeled.
24. (Amended) ~~A method for determining risk of colorectal cancer in a subject, comprising the steps of:~~
 - a. The method of claim 21, wherein determining whether an allele of the MnSOD gene in the subject comprises a mutation in the coding region for the MTS comprises determining the base identity of a portion of genomic DNA from ~~the subject's cell a~~ sample from the subject, said genomic DNA comprising an MnSOD gene comprising a coding region for the mitochondrial targeting sequence, said portion corresponding to position 351 as defined in SEQ ID NO:1 of said MnSOD gene in said mitochondrial targeting sequence; and
 - b. ~~correlating said base identity with a risk for colorectal cancer.~~
25. (Amended) The method of claim 24; wherein the base identity of ~~position 351 said~~ portion is determined by sequencing ~~a portion of said mitochondrial targeting sequence of said MnSOD gene containing said position 351.~~
26. (Amended) The method of claim 24; wherein the base identity of said portion ~~position~~ 351 is determined by digesting said portion ~~of the mitochondrial targeting sequence of said MnSOD gene with an appropriate~~ restriction endonuclease ~~appropriate to~~ determine the base identity of said ~~position 351.~~

Appendix I—MARKED UP VERSION

27. (Amended) The method of claim 24; wherein said base identity is determined by examining an RNA fraction from said subject's cell sample, whereby the identity of said genomic DNA at said position 351 can be determined.
28. (Amended) The method of claim 24; wherein the mutation in the coding region for the MTS resulting in a loss of α -helical structure in the MTS is a risk for developing colorectal cancer is assessed to be greater than that of the unaffected relevant population when the base identity a C at said position 351 is homozygous for C.
- 31. (New) A method of determining relative age-related risk of colorectal cancer in a subject susceptible thereto, comprising:
- determining whether a first allele of a manganese superoxide dismutase (MnSOD) gene in the subject comprises a mutation in the coding region for the mitochondrial targeting sequence (MTS) of the MnSOD protein resulting in a loss of α -helical structure in the MTS;
- determining whether a second allele of the MnSOD gene in the subject comprises a mutation in the coding region for the MTS of the MnSOD protein resulting in a loss of α -helical structure in the MTS;
- assigning a lower risk of developing early onset colorectal cancer to a subject having no mutation in either the first or second allele of the MnSOD gene resulting in a loss of α -helical structure in the MTS;
- assigning a higher risk of developing early onset colorectal cancer to a subject having mutations in both the first and second alleles of the MnSOD gene resulting in a loss of α -helical structure in the MTS; and
- assigning an intermediate risk of developing early onset colorectal cancer to a subject having a mutation in only one of the first and second allele of the MnSOD gene resulting in a loss of α -helical structure in the MTS.—